THE EFFECTIVENES OF DEMINERALIZED BONE MATRIX AUGMENTATION ON RABBIT MANDIBULAR OSTEOBLAST DENSITY AFTER INCISOR EXRACTION

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Abstract

The bone defect due to tooth extraction can be preventively managed by adding powder of bone graft intended for augmentation which eventually induces the formation of new bones. This wound healing in hard tissue is preceeded by the presence of osteoblast which is the bone lining cell responsible for the production of the bone matrix constituents. The objective of this study was to determine the effectiveness of the powder of demineralized bone matrix (DBM) after extraction of incisor in the rabbit alveolus mandibular bone on the osteoblast density. Twenty four male rabbits aged 2.5 to 3.0 months weighed 900 to 1,100 grams were randomly divided into two groups. The treated rabbits were augmented with DBM after the incisor extraction on mandible. The mucosa was then sutured. On the other group, the controlled rabbits received similar treatments with those of the treated rabbits except there was no augmentation of DBM. Decapitation of treated and controlled rabbits was made on day 5, 7, 10, and 14 days post surgery, each with three rabbits. Mandibles were cut, decalcified, and planted in paraffin block. The staining was done using HE. Significant differences in the density of osteoblast were noted on day 5, 7, 10 and 14 post surgery, indicating that powder DBM successfully generates the new bone by osteoconduction. *Indonesian Journal of Dentistry 2006; Edisi Khusus KPPIKG XIV:297-302*

Keywords: osteoblast, demineralized bone matrix, augmentation, osteoconduction

Introduction

Transplantation purports to reconstruct the defect parts of body inflicted by diseases and trauma. Bone grafting is often essential in providing the best possible set of conditions for the anchorage of implants or augmentation in areas lacking in quality or quantity. The type of mostly used in practice is allograft mainly due to its capability of being produced abundantly in various forms and sizes. Two salient functions of bone-graft are to hasten the process of osteogenesis and to provide mechanical support for recipient skeletal. According to Darwono documents that osteogenesis can happen through three methods: a) Graft surface cell – the bone still alive at the time of being moved then splits itself and establishes a new bone. This mechanism can prevail on *cancellous autograft*, but sometimes happen on *fresh cortical graft. b) Osteoinduction* is the process of pulling the recipient's pluripotential cells, found in the bone-graft surrounds. It works because bone-graft contains the mediator of osteoinduction, that are Bone morphogenic protein (BMP) and osteogenin. BMP basically is non-species specific hydrophilic glycoprotein, which is bone matrix; c) *Osteoconduction* is the process of graft resorption, which is then changed into the recipient's new bone gradually.[1] This wound healing

process is undergone through several stages, that are inflammation, revascularisation, osteogenesis, remodelling, and then the formation of new bone that is mechanically strong.

It has been reported by Mercier [2] that the abnormality of bone in the realm of oral surgery is bone defect, in most cases engendered by tooth extraction. Either the maintenance or the prevention of bone defect can be done by giving bone-graft for augmentation in order to induce the growth of new bone.[3,4]

Allograft in demineralized bone matrix powder form comprises collagenous and non-collgenous. As reported by Urist that BMP could be extracted from the organic component of bone and he determined that the BMPs are embedded in bone matrix.[5] Ludwig *et al*,[6] stated that there were other growth factors such as platelet derived growth factor (PDGF) and transforming growth factor-beta (TGF-β). This powder allograft is able to show conductive matrix, inductive cells, and other factors that in physiologically normal heal the condition.[7] Demineralized bone matrix (DBM) is a freeze dried allograft that can be restored at room temperature; its biochemical nature declines since the drying process uses freeze drying mechanism and the sterilisation utilises γ (gamma)ray. This graft has an advantage of reducing the immune reaction and having easily-applied form. The use of this type of allograft has been reported by Wilkins and Stringer[8] as an effective grafting material with relatively low complication risk. DBM powder is capable of providing maximum surface required to interact with target recipient's cells yielding best results for bone induction.[9] The powder that has particle sized 420-850 μm will yield very good results of osteoinduction.[10]

The function of osteoblast is to synthesise the organic components of bone matrix, that are type I collagen, proteoglycan, and glycoprotein. The addition of inorganic materials of bone hinges on the availability of live osteoblast. If the osteoblast is actively involved in the establishment of matrix, the shape will range from cuboids to cylindrical, with cylindrical cytoplasm. In the staining of Haematoxylin Meyer Eosin (HE), the osteoblast lies on the surface of bone tissue, appearing side by side similar to that in a layer of epitel.[11] The osteoblast produces TGF- β1 that can stimulate the formation of new bone and prevent the tissue degradation.[12] The aim of this study was to determine the effectiveness of DBM augmentation after incisivus extraction in rabbit alveolus mandibular bone on the osteoblast density

Materials and Method

This study was done pure experimentally using post- test only group design. Twenty-four male rabbits aged 2.5-3 months with weights of 900-1,100 grams were randomly divided into two groups. The first treated group I was augmented by powder DBM (370-710μm) post-incisivus extraction, whilst the other group was not augmented, hence used as a controlled group. DBM in the form of allograft made from cortical bone of rabbit was prepared by the Bank of Tissue of RSUD Dr. Soetomo, Surabaya. The rabbits were injected with intra muscular with phenobarbital sodium based on 100mg/kg body weight; then after they were calm and sleepy, they were injected with Pehacain 0,2 ml on incisivus tooth labial of mandible. After the extraction ended, the wounds were cleaned by using sterilized cotton, and gingival were pressed to control bleeding problem. Subsequently, they were immediately augmented by DBM that has been mixed with the solution of lactate ringer. DBM was pressed to get in and fill the tooth sockets on mandible. Mucosa crease was then sutured by non-absorbable silk suture No. 5 (Ethycon silk suture). Analgesic 250 mg was also included into the liquid of *ad libitum* 250 ml for two days after surgery only. The food given was pellet (Japfa Comfeed) and aquadest.

On days 5, 7, 10 and 14 post-surgery, three rabbits from each group were randomly taken to be decapitated. Mandible as wide as incisivus tooth was cut and submerged into the solution of Lylis as the fixative solution and decalcification for 3-5 days. Then the process was followed by bone submerge into 70% of alcohol for 3 days. Subsequently, the bone was vertically cut in line with tooth axis using microtome and was imbedded in paraffin block. Furthermore, the tissue was again cut as wide as 6μm and put on object glass and processed for HE staining. The observation on osteoblast density was conducted on ten view fields each sample under light microscope with the 4x 100 objective by computing the number of osteoblast.

Results

Table 1 shows the mean and standard deviation of osteoblast density in alveolus mandibular bone in each group. The pattern of the density of bone's osteoblast is described in the two groups (Figure 1). However, overall the group of augmented rabbits is inclined to have more osteoblast. In the treatment

group, the density appears to be similar and has comparable number of osteoblast on day 7 and 10 post-surgery, but then increases on day 14. This phenomenon is of difference from that of controlled group, which on day 7 post-surgery still shows similar osteoblast density to that on day 5. The enhancement of osteoblast density can be seen 10 days after the surgery, and experiences a little increase on day 14. On day 7 after surgery, we found that sample No 16 of control group only has a little bone matrix. The same case was found in sample No 35 even on day 14 post-surgery. This phenomenon was different on group which were augmented with DBM appears denser of bone matrix (Figure 2).

Table 1. Mean and Standard Deviation of Osteoblast Number in Alveolus of Rabbit Mandible after Tooth Extraction Based on Day of Observation of Control and DBM Groups

n	Day after implantation	MEAN ± SD Control	MEAN± SD DBM
30	5	37,86 ± 6.14	39,43 ± 12,84
30	7	32,16 ± 25,74	53,93 ± 12,24
30	10	37,36 ± 24,87	56,43 ± 15,86
30	14	53,13 ± 17, 04	61,96 ± 23,72

DBM = powder of demineralized bone matrix

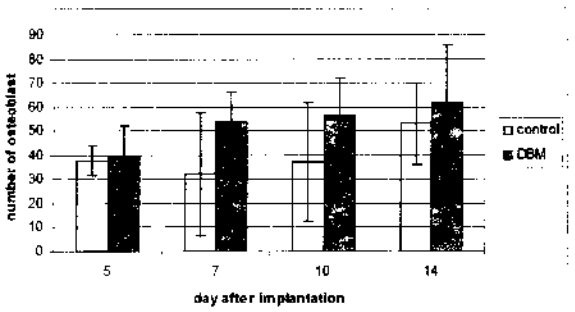

Figure 1. The growth of the osteoblast number in alveolus of rabbit mandible based on day of observation of control and DBM groups

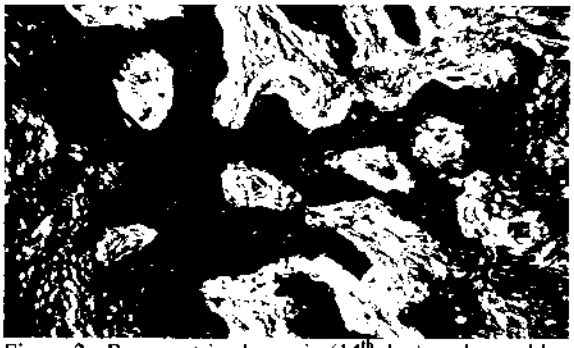

Figure 2. Bone matrix shown in (14th day) as denser blue stained on DBM group sample (400x)

Subsequently, Table 2 summarises the statistical analysis using Student t-test. The results indicate a difference significance of the osteoblast density between the two groups on all treatment days post-surgery. Hence, DBM shows its capability of being a osteoconductive material.

Tabel 2. T-Test of Osteoblast Number within Groups of Treatment Based on Observation Day

Time after augmentation	Significance
Day 5	0,027**
Day 7	0,001**
Day 10	0,011**
Day 14	0,025**

Discussion

This study's result indicates that DBM powder of cortical allograft can conduct the osteoblast cells density stimulation, the preliminary stage of new bone formation in mandibular alveolus post tooth extraction. The finding still does not totally correspond with previous research that shows DBM's capability of inducing and conducting the bone formation stimulation.[13, 14] In this study, the finding histologically shows a significant osteoblast density specifically on day 5, 7 , 10 and 14 in the DBM-augmented group. This result is in line with the study of Reddi *et al* [15] stated that the growth of the new osteoblast prevails between day 10 and 12 in the process of bone induction of DBM implantation.[15] During the second week, collagen bundle appears growing solidly on the wounded areas, and the bone formation process continues [16]. This fact substantiates the previous studies revealing

that cartilage histologically will experience mineralization on day 7-14 in the wake of the DBM implantation and will be replaced by new bone.[17, 18]

The mineralization process occurring in hard tissues of the body relies upon this extracellular matrix. This study's results indicate a significant difference of collagen fibre density on day 10 and 14 after the tooth extraction of previous study.[16] Collagen fibres on mandibular alveolus are mostly type I and II. This condition is in line with the study of Reddi *et al*[15] reporting that the synthesis of collagen type I prevails between day 10 and 12 in the process of bone induction of DBM implantation. Concurrently, osteoblast with intense basophile appears to be close to the vascular endothelium, and new bone is formed by appositional growth on the surface of the calcified matrix and the implanted nonliving collagenous matrix.[15] The osteoblast is the bone lining cell responsible for the production of bone matrix constituents, collagen and ground substance. Osteoblast is always found in the cluster of cuboidal cells along the bone surface. Under the light microscope used in this study, the osteoblast is morphologically characterized by a round nucleus at the base of the wall, and intensely basophilic cytoplasm.

DBM in this study, functioning as an augmentation material, indicates its capability of hastening the bone reconstruction by conducting the stimulation osteoblast formation that will be a new bone. The demineralized form of allograft bone is essentially a collagen fibre that reinforces the hydroxyapatite matrix containing active bone morphogenic proteins (BMP). The demineralized allograft tissue is fully incorporated in a patient's tissue by a well-established biological mechanism. The demineralization process exposes the BMPs, making them accessible to the surrounding cells.[19] However, as this study is only focused on 14 days histological observation, and also does not observe the phenomenon in molecular, DBM is heretofore seen to be indicating the osteoconductiveness. Accordingly, the formation of new bone is hypothetically to be achieved by the growth of the host cell in the DBM granules functioning as scaffold. This hypothesis is supported by Groeneveld and Burger,[20] also Strates and Tiedeman[21] revealing that histologically a new bone is formed more by osteoconduction than by osteoinduction, and the DBM plays a role more as scaffold than as *de novo* bone differentiation. Osteoconduction describes bone formation by the process of growth of capillaries and osteoprogenitors cells from the recipient bed into, around and through a graft or bio-implant. Therefore, the powder allograft DBM in this study acts as a scaffold for a new bone formation.

One of the parameters of bone healing is the growth of new cells. Healing following injury involves a series of well orchestrated cell-cell and cell-macromolecular interactions. Subsequently, the effect of bone healing augmented by the allograft of DBM powder is possibly triggered by several growth factors that it contains. The contents are. amongst the others, BMP, PDGF, and TGF-β that synergically cooperate with local recipient cell in influencing the proliferation of cells involved in the bone healing. The pattern of bony healing is dictated by the host bed, vascular supply, oxygen tension and the stability of bone segments. BMPs are abundant in bone and are produced by several cell types, including osteoblast. According to Groeneveld and Burger[20], BMPs belong to the TGF-β superfamily, consisting of a group of peptide growth factors. The BMPs are differentiation factors, causing mesencymal cells to differentiate them into bone- and cartilage forming cells.[19, 22] BMPs comprise dimmers interconnected by seven disulphide bonds;[23] this dimmerisation is a prerequisite for bone induction.[24] BMPs-2 and -4 form one subgroup, and have shown osteoinductivity with an identical mechanism as observed after ectopic implantation of osteoinductive DBM.[25] BMP-2 was also found to be chemotactic for mature osteoblast.[26] Inflammatory cell infiltration has been reported to decrease after implantation by addition of BMP.[27]

The effects of polypeptide growth and differentiation factors (PGDFs) mechanisms have been shown to have pleitropic on wound repair in nearly all tissues on the repair and regeneration of tissues.[22, 28] The expression of various GDFs following bone and soft tissue injury may regulate the repair and or regenerative process. While acidic and basic FGF show that both are found in bone matrix.[29] This bFGF is produced locally in bone during the initial phase of fracture healing and is known to stimulate cartilage and bone forming cells.[30] And in vitro where both forms stimulate DNA synthesis and cell replication.[31] It was reported that of significance, the angiogenesis which is a critical for the vascular invansion of bone potently stimulated by bFGF.[32]

Previous studies have shown that TGF-β1 has appeared to be a strong promoter of extracellular matrix production in many cell types and tend to be highly dependent upon bone cell source and local environment.[33] The binding of TGF-β1 to cell

receptors mediate stimulation of osteoblast and hematopoiteic stem cells mitosis. TGF-β1 stimulates osteonectin biosynthesis as well as bone matrix deposition and chemotaxis and may act as a bone coupling factor linking bone resorption to bone formation.[34] The powder DBM used in this study show that this graft has good osteoconductive properties which is desirable for the restoration of the bone function. Bone reconstruction is best understood if the process of bone healing is first considered.

Conclusion

Demineralized bone matrix (DBM) augmented in alveolus of mandibular post incisivus extraction has proven its effectively of significantly stimulating the density of osteoblast and indicating it was successfully generates the new bone by osteoconduction. The growth of osteoblast per se is of significant differences on day 5, 7, 10 and 14 after the existence of wounds.

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